

Urban Particle-induced Apoptosis and Phenotype Shifts in Human Alveolar Macrophages

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Epidemiological studies report a small but positive association between short-term increases in airborne particulate matter and small increases in morbidity and mortality from respiratory and cardiovascular disease in urban areas. However, the lack of a mechanistic explanation to link particle exposure and human health effects makes it difficult to validate the human health effects. The present study tested the hypothesis that urban particles could cause apoptosis of human alveolar macrophages (AM) and a shift of their phenotypes to a higher immune active state, which would provide a mechanism to explain an inflammatory response. Freshly isolated human AM were incubated for 24 hr with urban particles (#1648 and #1649), Mount Saint Helen's ash (MSH), and residual oil fly ash (ROFA). Cell viability was assessed by trypan blue exclusion and apoptosis was demonstrated by morphology, cell death ELISA, and DNA ladder formation. Additionally, AM were characterized according to RFD1⁺ (immune stimulatory macrophages) and RFD1⁺7⁺ (suppressor macrophages) phenotypes by flow cytometry. ROFA particles caused AM necrosis at concentrations as low as 10 µg/ml, urban particles had no effect except at 200 µg/ml, and MSH had no effect at 200 µg/ml. ROFA (25 µg/ml) and particles #1648 or #1649 (100 µg/ml) caused apoptosis of AM by all three criteria, but 200 µg/ml MSH had no effect. Finally, 25 µg/ml ROFA and 100 µg/ml particles #1648 or #1649 up regulated the expression of the RFD1⁺ AM phenotype, while only ROFA decreased the RFD1⁺7⁺ phenotype. Consequently, ROFA and urban particles can induce apoptosis of human AM and increase the ratio of AM phenotypes toward a higher immune active state (i.e., increased RFD1⁺:RFD1⁺7⁺ ratio). If urban particles cause similar changes *in vivo*, this could result in lung inflammation and possible increased pulmonary and cardiovascular disease. **Key words:** inflammation, macrophage subpopulations, RFD1, RFD7, ROFA. *Environ Health Perspect* 106:127–132 (1998). [Online 23 January 1998] <http://ehpnet1.niehs.nih.gov/docs/1998/106p127-132holian/abstract.html>

Epidemiologic studies suggest that preexisting disease increases the susceptibility of individuals to the effects of small increases in urban particle air pollution (1,2). However, these studies provided little information on the health status or other relevant circumstances (i.e., personal exposures) of the populations affected since abstracted death certificates or hospital discharge diagnostic summary data were used to ascertain health status or cause of death. More recent prospective cohort studies (3–5) suggested that long-term exposure to particle air pollution may be associated with an increase in the morbidity and mortality from respiratory and/or cardiovascular diseases in the general population. Major questions remain as to the specific size fraction, chemical component(s), and biologically relevant exposure metric that would cause the observed effects. Further, these results remain controversial, in part, because the responsible mechanisms are unknown that would explain these effects.

One potential mechanism to explain the health effects of urban particles may involve their interactions with the alveolar macrophage (AM). The AM is the central lung cell in the regulation of the immune response to inhaled pathogens and the development of inflamma-

tion (6). Additionally, the AM is central to nonspecific host defense through phagocytosis of foreign material, such as urban particles (6).

In order to examine potential mechanisms that may account for urban particle-induced lung injury, a number of models have been used. For example, particulate matter collected by the EPA during the middle to late 1970s has been shown to produce a variety of effects on the lung using *in vivo* and *in vitro* models. Urban airborne particulate matter collected in St. Louis, Missouri (#1648), and Washington, D.C. (#1649), using a specially designed baghouse (7), has been recently shown to stimulate AM to release cytokines, but most of the activity appeared to be accounted for by endotoxin contamination (8). Alternatively, residual oil fly ash (ROFA) particles have been reported to cause lung inflammation in a rat model (9). However, these particles did not stimulate release of inflammatory cytokines when cultured *in vitro* with AM (8). Therefore, it is likely that some mechanism other than direct stimulation of AM release of cytokines may be involved in the development of lung inflammation by these types of particles.

Several studies have demonstrated the presence of distinct AM phenotypes within

the AM pool obtained by bronchoalveolar lavage from human subjects. The macrophage phenotypes have been described on the basis of expression of RFD1⁺ and RFD7⁺ surface epitopes (10,11). RFD1⁺ and RFD7⁺ correspond to a 28–33 kDa protein within the Major Histocompatibility Complex II and a 77 kDa protein, respectively (11). Using these markers, Spiteri et al. (12,13) and van Haarst et al. (14) classified RFD1⁺ AM as those that strongly stimulate T lymphocytes in allogenic mixed lymphocyte reactions, contain low amounts of intracellular fluorescent material, and are dendritic-like, nonadherent to glass, and weakly phagocytic. RFD7⁺ AM are mature phagocytic cells (13) and RFD1⁺7⁺ AM function as suppressor cells (13,14). Both RFD7⁺ and RFD1⁺7⁺ AM have significant amounts of intracellular fluorescent material suggesting phagocytic activity. RFD1⁺7⁺ cells stimulate allogenic mixed lymphocyte reactions poorly and actively repress the T-cell stimulatory capacity of RFD1⁺ AM (13,14). Further, addition of RFD1⁺ monoclonal antibody inhibits RFD1⁺ AM-mediated T-cell stimulatory activity (15). Studies of patients with various inflammatory diseases suggest that shifts in AM phenotypes (higher RFD1⁺:RFD1⁺7⁺ ratio) could play a role in disease progression (14,16,17). Consequently, regulation of these phenotypes could prove to be an important source of altering disease progression and/or outcome. Therefore, if particles induce a shift in these populations to a higher ratio of RFD1⁺:RFD1⁺7⁺ cells, inflammation may develop or be exacerbated in individuals with an already abnormal phenotype ratio.

A potential mechanism to account for a shift in the AM phenotype ratio may be apoptosis of the suppressor population. Particles that induce inflammation such as asbestos and silica, but not nonfibrogenic particles such as titanium dioxide and wolastonite, have been reported to cause human AM apoptosis (18,19). Apoptosis

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occurred at concentrations of these particles well below those causing other forms of cell injury and below doses causing human AM to release inflammatory cytokines (20). In addition, another inflammatory agent, bleomycin, has been shown to be a potent inducer of apoptosis in human AM (21). Therefore, apoptosis of suppressor AM could increase the stimulatory (RFD1⁺) to suppressor (RFD1⁺7⁺) macrophage ratio.

The present study tested the hypothesis that urban particles could cause apoptosis of human AM and shift their phenotypic ratio. Freshly isolated human AM were incubated with well-characterized urban particles (#1648 and #1649), negative control Mount Saint Helen's ash (MSH), and ROFA. The AM were then examined for cell viability by trypan blue exclusion and for apoptosis by morphology, cell death ELISA, and DNA ladder formation. Finally, the AM RFD1⁺ and RFD7⁺ phenotypes were characterized by flow cytometry.

Materials and Methods

Cell cultures. Human alveolar macrophages were obtained by bronchoalveolar lavage of normal, nonsmoking adult volunteers of either sex as previously described (20). This study has been approved by The University of Texas Houston Health Science Center Committee on Human Subjects, and all volunteers signed an informed consent form. Instillations of 240–300 ml sterile saline resulted in recoveries of 200–260 ml lavage fluid that was kept at 4°C until cells were isolated from the lavage fluid by centrifugation. The saline supernatant was aspirated and discarded, and the cell pellet was resuspended in a small volume (1–5 ml) of HEPES buffered medium 199 (Gibco BRL, Gaithersburg, MD) with 10% fetal bovine serum (FBS; Sigma, St. Louis, MO), and antibiotics (50 U/ml penicillin, 50 µg/ml gentamycin, and 50 µg/ml streptomycin).

The cell count was determined with a ZBI Coulter Counter (Coulter Electronics, Hialeah, FL). Lavages yielded an average of 2×10^7 cells that were >92% alveolar macrophages, as verified by Leukostat staining (Fisher Scientific, Houston, TX). Viability was >90% as determined by trypan blue exclusion.

AM were cultured at 1×10^6 cells/ml at 37°C in the media described above for 24 hr. Cell cultures were maintained in suspension by slow end-over-end tumbling (Labquake Shakers; Labindustries, Berkeley, CA) in sterile polypropylene tubes (PGC Scientific, Gaithersburg, MD) at 37°C in a water-jacketed CO₂ incubator (Queue, Parkersburg, WV) (22).

Particulate matter. ROFA particles were kindly donated by Dan Costa and Kevin Dreher from the EPA. ROFA are emission from a stationary source. Fugitive (i.e., those leaving the source) ROFA were collected on a Teflon-coated glass fiber filter placed downstream of the cyclone of a power plant burning low-sulfur residual oil, as previously described (9). Urban particulate matter #1648 and #1649 were obtained from the National Institute of Standards and Technology (Gaithersburg, MD). Volcanic MSH was collected and provided by Andrew McFarland, Texas A&M University. The characteristics of these particulate matter samples have been described by a number of investigators (7,8,23–26). A summary of the main physical and chemical properties of the four types of particulate matter is presented in Table 1. ROFA, #1648, and #1649 have similar physical diameter distribution characteristics, while MSH particles are larger. Both #1648 and #1649 particles are enriched in organic carbon content as compared to ROFA and MSH. The inorganic fraction of ROFA particles is also far more soluble than for MSH and has a lower pH (24). From the stand-

point of elemental composition, ROFA particles are enriched in elements present in residual and crude oils, while being relatively poorer (particularly as compared to MSH) in crystal-origin elements. The airborne particulate matter samples show a mix of crustal- and fuel-burning-derived elements. The soluble transition metal-enriched fraction of ROFA particles has been implicated in a number of acute effects on the lung by previous investigators (24).

Cell viability assays (trypan blue exclusion). Cells in culture suspension were exposed to trypan blue dye [0.04% in phosphate-buffered saline (PBS)], placed on a hemocytometer, and examined under light microscopy. Two hundred cells were counted at random after each treatment, and the percentage of blue cells was expressed as the percentage of nonviable cells for any given condition.

Apoptosis assays. Macrophage apoptosis was examined by a combination of Leukostat staining and detection of DNA fragmentation in the cells. For Leukostat staining, cells were suspended in PBS (pH 7.2) at room temperature, cytocentrifuged onto positively charged microscope slides (Fisher Scientific) at 1500 rpm for 5 min, fixed in cold methyl alcohol for 5 min, stained in Leukostat eosin stain for 2 min, and then stained in Leukostat methylene blue stain for 4 sec. The slides were dried in air and examined by light microscope at 630× (dry objective). Apoptotic cells have condensed, darkly stained nuclei, which may be fragmented. In addition, apoptotic cells are smaller in size than healthy cells.

For the detection of oligonucleosomes in cytoplasmic fractions of the cells, the samples were processed and analyzed using the Cell Death Detection ELISA kit (Boehringer Mannheim, Indianapolis, IN) according to the manufacturer's protocol. The assay is based on the quantitative sandwich-enzyme-

Table 1. Physical and chemical characteristics of residual oil fly ash (ROFA), #1648, #1649, and Mt. St. Helen's ash (MSH) particles

Characteristic	ROFA	#1648	#1649	MSH
Geometric diameter (µm)				
Count median (range) ^a	0.5 (0.29–0.9)	0.4 (0.2–0.8)	0.4 (0.2–0.8)	1.4 (0.7–2.2)
Volume median ^a	1.1	1.4	1.1	2.3
Mass median aerodynamic diameter (σ _g) ^b	1.95 (2.19)			
Solubility (%)	96 ^b			Very low ^c
Organic carbon (%)	1.2 ^b	4–7 ^d	5.7 ^d	<1 ^d
Inorganic fraction (%)	91.2 ^b		60.1 ^e	
pH	2.4 ^f			6.3 ^f
Principal elements (>1% w/w)	S, V, Ni, Fe, Na, Si, Mg ^b	Si, S, Fe, Al, K ^g	Si, S, Fe, Al, K ^g	Si
Trace elements (>0.01%–<1% w/w)	Al, K, Ti, P, Mn, Pb ^b	Mg, Pb, Na, Zn, Cl, Ti, Cu, As, Cr, Ba, Br, Mn ^g	Mg, Pb, Na, Zn, Cl, Ti, Cu, As, Cr, Ba, Br, Mn ^g	Ca, Mg, Na ^a

^aAdapted from Hamilton et al. (27).

^bDerived from Dreher (24).

^cAdapted from Hatch et al. (23).

^dAdapted from Becker et al. (8).

^eAdapted from May et al. (7).

^fAdapted from Hatch et al. (25).

^gAdapted from Hatch et al. (23), Dreher et al. (24), and the National Institute of Standards and Technology (26).

immunoassay principle using monoclonal antibodies directed against DNA and histone. For each sample, 1×10^5 cells were processed, 5,000 cells were used for each reaction, and triplicate reactions were performed for each sample. The results are expressed as optical density (OD) measured at 405 nm, and the results are reported as the mean \pm standard error (SE) of the OD values.

For detection of DNA internucleosomal fragmentation formation, human AM were cultured for 24 hr as described above and washed once with PBS before DNA isolation. Genomic DNA was isolated by using the DNA Isolator (Genosys, Woodlands, TX) according to the manufacturer's protocol. The isolated genomic DNA was dissolved in 10 mM Tris-1 mM EDTA buffer, pH 8.0, and 3'-end labeled with α - 32 P dCTP (ICN Pharmaceuticals, Costa Mesa, CA) by incubation of 1 μ g of DNA in 50 μ l reaction buffer [50 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 200 μ M dATP, 200 μ M dGTP, 200 μ M dTTP, 2 μ l α - 32 P dCTP, and 2 U DNA polymerase and large fragment (klenow)] at 37°C for 30 min. The α - 32 P dCTP-labeled DNA was mixed with 10 μ l loading buffer (0.25% bromophenol blue, 100 mM EDTA, and 30% glycerol). The same amount of α - 32 P dCTP-labeled DNA (50 ng) was loaded onto a 2% agarose gel and run at 5 V/cm for 5 hr in 40 mM Tris-acetate buffer, pH 8.0, with 1 mM EDTA. The gel was dried at 80°C under vacuum in a gel dryer and exposed to X-ray film for detection of resolved labeled DNA fragments.

Immunostaining. At the termination of cell culture at 24 hr, all cultures were centrifuged in an Eppendorf Microcentrifuge 5415 C (Brinkman Instruments, Germany) at maximum speed ($12,000 \times g$) for 20 sec. The culture media was aspirated and the cell pellet (0.5×10^6 cells) was resuspended in 500 μ l PBS with 3.5% bovine serum albumin (BSA). The monoclonal antibodies to RFD1 (murine IgM) and RFD7 (murine IgG1) surface antigens (Serotec; Kidlington, Oxford, England) were added concomitantly at a 1:200 dilution (2.5 μ l in 500 μ l). This mixture was incubated for 30 min at room temperature. This incubation was terminated with centrifugation and aspiration. The cell pellet was then washed three times in PBS. The cell pellet was again suspended in PBS/BSA buffer, and the fluorescein anti-mouse IgM and the R-phycoerythrin anti-mouse IgG (Vector Laboratories, Burlingame, CA) were added concomitantly at a 1:100 dilution (5 μ l in 500 μ l) and incubated 30 min at room temperature. This incubation was terminated and the cell pellet was washed three times in PBS as described above. The cell pellet was then suspended in 1% formaldehyde (PBS buffered) and stored

at 4°C prior to flow cytometric analysis. Flow cytometry was performed on a FACScan flow cytometer (Becton-Dickinson) using Consort 30 software. Using forward and side scatter of the total cell population, gates were drawn to include macrophages based on size and granularity of the cells. The instrument was calibrated with fluorescein isothiocyanate (FITC)- and phycoerythrin (PE)-coated beads to compensate for any overlap within the green and red fluorescence wavelengths. Cells stained without the inclusion of primary antibodies resulted in no significant staining.

Statistical analyses. For cell viability and cell death ELISA data, values are presented as mean \pm SE. The number of individuals whose cells were used for a given experiment, denoted by n , is provided in the figures. For each experiment, statistical treatment included a one-way analysis of variance (ANOVA) followed by a Student Newman-Keuls test for post hoc pair-wise comparison (significant difference compared to control; $p \leq 0.05$). Due to individual variability, the marker data were normalized for each subject by dividing the particle-stimulated value (% of cells) by the control value (% of cells), and a one-tailed t -test was applied for each particle type using one-sample hypothesis testing and assuming the population mean equaled 1.

Results

Effects of particles on viability of human alveolar macrophages. To determine if any of the particles examined had cytotoxic effects on human AM *in vitro*, cell viability was quantitated by trypan blue exclusion after 24-

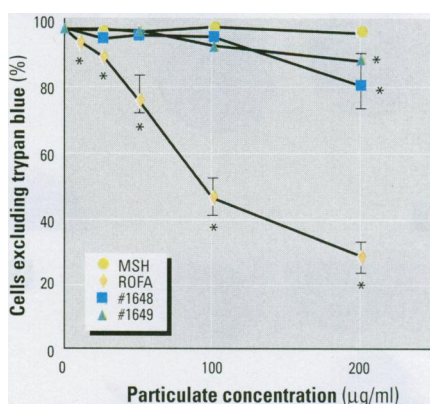


Figure 1. Effect of particles on human alveolar macrophage viability. Human alveolar macrophages were incubated with varying concentrations of residual oil fly ash (ROFA), Mount St. Helen's ash (MSH), and urban particles #1648 and #1649 for 24 hr at 37°C as described in Materials and Methods. Cell viability was examined by trypan blue exclusion. The data represent the mean \pm standard error ($n = 6$ subjects).

*Denotes a significant decrease ($p < 0.05$) in cell viability compared to control cells.

hr incubations with varying concentrations of particles, as shown in Figure 1. MSH had no effect on AM viability at any concentration examined. The macrophage responses to both urban dusts (#1648 and #1649) were similar, with no effects at or below 100 μ g/ml, but with a significant decrease in cell viability at 200 μ g/ml. In contrast, ROFA particles were highly cytotoxic, and significant decreases in cell viability were observed at concentrations as low as 10 μ g/ml.

Effects of particles on apoptosis of human alveolar macrophages. Since we previously demonstrated that pro-inflammatory particles induced apoptosis of human AM at concentrations below those causing necrosis (18,19), the effects of ROFA and urban dusts on human AM apoptosis were examined using multiple approaches. Cell death ELISA quantitates apoptosis through the measurement of cytosolic histone-bound DNA fragments formed during apoptosis. Results from this assay are shown in Figure 2. MSH had no apoptotic effects at any concentration examined (up to 200 μ g/ml). An increase in histone-bound DNA fragments was evident at 100 μ g/ml of both urban dusts, which became significant at 200 μ g/ml. In contrast, ROFA particles induced significant apoptosis at concentrations as low as 25 μ g/ml. A decrease in the cell death ELISA for ROFA was evident at 200 μ g/ml compared to that observed at lower concentrations. This is similar to results from other studies in which a decrease in the cell death ELISA was observed when there was extensive cytotoxicity (27). Because 100

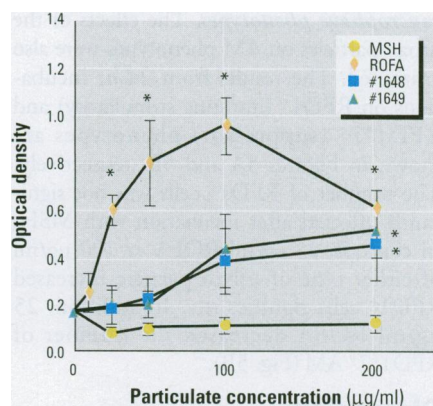


Figure 2. Effect of particles on human alveolar macrophage apoptosis measured by cell death ELISA. Human alveolar macrophages were incubated with varying concentrations of residual oil fly ash (ROFA), Mount St. Helen's ash (MSH), and urban particles #1648 and #1649 for 24 hr at 37°C as described in Materials and Methods. Apoptosis by cell death ELISA was measured as described in Materials and Methods. The data represents the mean \pm standard error ($n = 6$ subjects).

*Denotes a significant increase ($p < 0.05$) in cell apoptosis compared to control cells.

$\mu\text{g/ml}$ urban particles was not cytotoxic and it appeared to induce apoptosis (lowest observed effect level), this concentration was used for the remaining studies. For the additional studies, 25 $\mu\text{g/ml}$ ROFA (a highly apoptotic concentration) and 200 $\mu\text{g/ml}$ MSH (negative control) were selected.

To confirm that the particles caused apoptosis, human AM were examined morphologically. Figure 3 shows the outcome of 24-hr incubations with the particles at the concentrations described above. AM appearance was normal in the untreated (Fig. 3A) and MSH-treated cells (Fig. 3B). In contrast, cells incubated with 25 $\mu\text{g/ml}$ ROFA (Fig. 3C), 100 $\mu\text{g/ml}$ #1648 (Fig. 3D), and 100 $\mu\text{g/ml}$ #1649 (Fig. 3E) all demonstrated morphological features characteristic of apoptotic cells including cell shrinkage, condensed darkly stained nuclei, and apoptotic bodies. In the final procedure to confirm apoptosis, the ability of the particles to induce the formation of a 180–200 base pair DNA ladder characteristic of apoptosis was examined (Fig. 4). Similar to the morphologic results, control AM and AM incubated with MSH demonstrated no visible DNA ladder formation. In contrast, incubations with 25 $\mu\text{g/ml}$ ROFA or 100 $\mu\text{g/ml}$ of either type of urban particle demonstrated the formation of DNA ladders characteristic of cells undergoing the cell death by apoptosis. Taken together, the evidence presented from the cell death ELISA, cell morphology, and DNA ladder formation demonstrate that ROFA and urban particles cause apoptosis of human AM.

Effects of particles on human alveolar macrophage phenotypes. The effects of the urban particles on AM phenotypes were also examined. The results from 24-hr incubations on RFD1⁺ (immune stimulatory) and RFD1⁺7⁺ (suppressor) phenotypes are shown in Figures 5A and 5B, respectively. The number of RFD1⁺ cells was not significantly affected after incubation with MSH. In contrast, 25 $\mu\text{g/ml}$ ROFA or 100 $\mu\text{g/ml}$ of either type of urban particle increased RFD1⁺ cells significantly. In addition, 25 $\mu\text{g/ml}$ ROFA decreased the number of RFD1⁺7⁺ AM (Fig. 5B).

Discussion

One of the difficulties in linking human exposure to urban particles and the reported health effects has been the lack of a mechanistic explanation. Previous studies examined the ability of these particles to induce the release of inflammatory cytokines, but with negative results (8). The current study suggests that ROFA and two types of urban particles induce apoptosis and induce shifts in the alveolar macrophage phenotypes to a

more immune stimulatory state, i.e., an increase in the ratio of RFD1⁺ to RFD1⁺7⁺ cells. Perhaps this relative increase in the immune stimulatory AM phenotype or RFD1⁺ AMs can activate T-helper (Th1) cells to release proinflammatory cytokines such as interferon- γ (IFN- γ). IFN- γ in turn will activate AM and initiate the inflammatory cycle (28). Alternatively, increased activity of the Th2 pathway (humoral pathway) would increase the release of interleukins 4 and 5, thereby potentially exacerbating asthma or asthma-like diseases (17,29).

The confirmation that ROFA and two

well-characterized types of urban particles induce apoptosis is based on three independent lines of evidence. First, #1648, #1649, and ROFA all caused a concentration-dependent increase in cytosolic histone-bound DNA fragments (cell death ELISA). The results from the cell death ELISA assay were confirmed by morphological evidence of apoptosis induced by ROFA and both types of urban particles. Finally, these three types of particles induced the formation of DNA ladders. Together, these three lines of evidence establish that ROFA and urban particles can cause apoptosis of AM. In contrast, MSH did not have any adverse

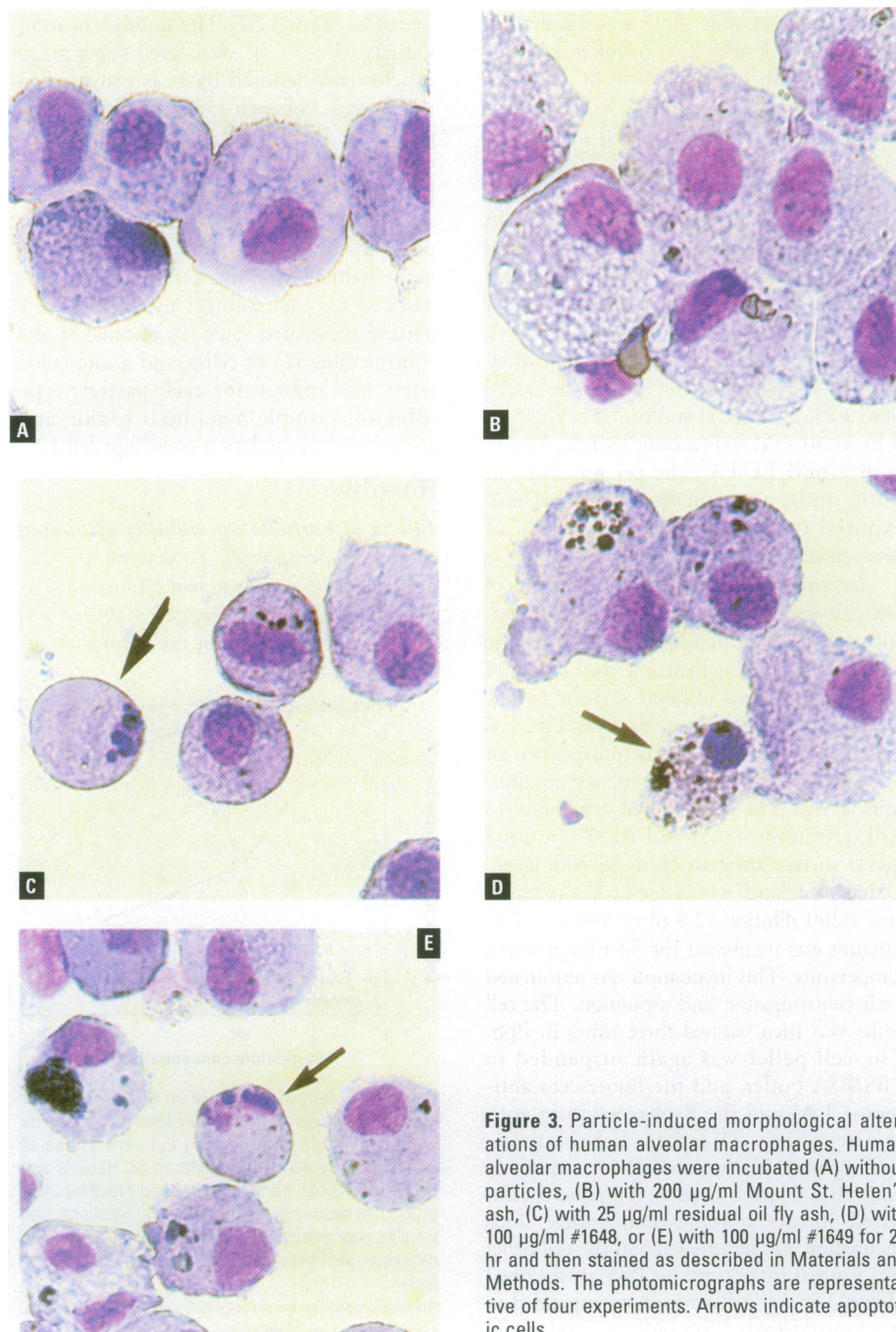


Figure 3. Particle-induced morphological alterations of human alveolar macrophages. Human alveolar macrophages were incubated (A) without particles, (B) with 200 $\mu\text{g/ml}$ Mount St. Helen's ash, (C) with 25 $\mu\text{g/ml}$ residual oil fly ash, (D) with 100 $\mu\text{g/ml}$ #1648, or (E) with 100 $\mu\text{g/ml}$ #1649 for 24 hr and then stained as described in Materials and Methods. The photomicrographs are representative of four experiments. Arrows indicate apoptotic cells.

effects on human AM.

Previous studies have demonstrated that AM apoptosis occurs at concentrations of a number of cytotoxic agents below those causing necrosis (18,19,21,27,30). The same appears true for the urban particles (#1648 and #1649) because apoptosis occurred at 100 $\mu\text{g}/\text{ml}$ without evidence of necrosis. The data are less compelling for ROFA particles since they caused necrosis at very low concentrations. In addition, ROFA particles were a much more potent inducer of alveolar macrophage apoptosis than urban particles, and on the basis of surface area, ROFA was equally if not more potent than crystalline silica or asbestos in inducing apoptosis (18,19). Further, at high concentrations of ROFA that induced extensive cytotoxicity, apoptosis decreased. These findings are consistent with recent studies that indicate the high inflammatory and fibrotic potential of ROFA (9,24). Preliminary studies using AM isolated from F344 and Sprague-Dawley rats indicate that the three types of particles also cause

apoptosis of rat AM and were biologically active at approximately twofold lower concentrations (data not shown).

It has recently been proposed that the high inflammatory potential of ROFA can be attributed to the soluble transition metal content of ROFA, in particular iron, nickel, and vanadium (24). These transition metals may also account for the high apoptotic potential of ROFA in our studies. Preliminary studies using nickel (II) and vanadium (V) demonstrated that low ($\leq 10 \mu\text{M}$) concentrations of either metal caused apoptosis of rat AM *in vitro* (data not shown). Therefore, the potential of the soluble metals to cause inflammation and apoptosis of AM were consistent and could be responsible for the bioactivity of urban particles because they also contain metals, but less than ROFA (23,24,26).

In addition to causing AM apoptosis, ROFA and urban particles were able to alter the ratio of AM phenotypes. ROFA and both types of urban particles approximately doubled the RFD1⁺ AM phenotype in 24-hr cultures (5% of the AM in control cultures are RFD1⁺). In contrast, only ROFA particles decreased the RFD1⁺7⁺ phenotype. ROFA particles were the most potent in inducing apoptosis; this would explain their potential to decrease the RFD1⁺7⁺ phenotype. On the other hand, urban particles were less active and may take longer to significantly deplete the RFD1⁺7⁺ subpopulation. Because the urban particles increased the expression of RFD1⁺ AM without an apparent decrease in RFD1⁺7⁺ AM, the urban particles may induce the expression of the RFD1⁺ AM phenotype. This observation suggests that the AM phenotypes are not static, but appear to be able to shift in response to extracellular factors. In support

of this hypothesis, recent studies (including preliminary studies from our laboratory) have demonstrated that human monocytes become RFD1⁺-like in the presence of IFN- γ or interleukin-4 and RFD7⁺-like in the presence of interleukin-10 (31). Therefore, urban particles may be able to up regulate RFD1⁺ AM expression without an apparent decrease in RFD1⁺7⁺ AM expression.

The two urban particulate matter samples (#1648 and #1649) were not distinguishable in any of the assays. This is not unexpected because these particles were obtained from areas with high mobile and point source emissions and their physical characteristics are similar (26). This finding is similar to other recent reports that demonstrate similar biological activity of these two types of particles (8). Further studies to examine the effects of particles obtained from significantly different sources and examination of particles of different sizes will be crucial to the understanding of the components on the particles that may contribute to the potential health effects. Smaller particles with a higher surface area (and possibly more metals per unit mass) may have greater apoptotic potential (24).

These studies used human AM and the results are directly applicable to humans, but the *in vivo* responses remain to be determined. Additionally, studies from a number of laboratories, including our own, indicate that the ratio of AM phenotypes varies in the normal population and is already shifted to a higher inflammatory ratio in individuals with a number of pre-existing inflammatory conditions (14,16). Therefore, an increased particle burden in susceptible individuals may induce an even more significant change in AM phenotype. This may, in part, explain the association

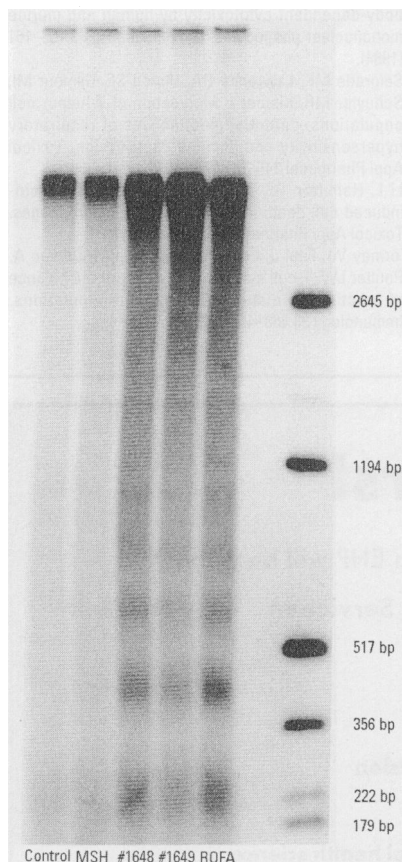


Figure 4. Formation of DNA ladders by residual oil fly ash (ROFA) and urban particles. Human alveolar macrophages were incubated without particles (lane 1), with 200 $\mu\text{g}/\text{ml}$ Mount St. Helen's ash (MSH) (lane 2), 100 $\mu\text{g}/\text{ml}$ #1648 (lane 3), 100 $\mu\text{g}/\text{ml}$ #1649 (lane 4), and 25 $\mu\text{g}/\text{ml}$ ROFA (lane 5) for 24 hr; DNA was then isolated and electrophoresed, as described in Materials and Methods. bp, base pairs. The result is representative of two experiments.

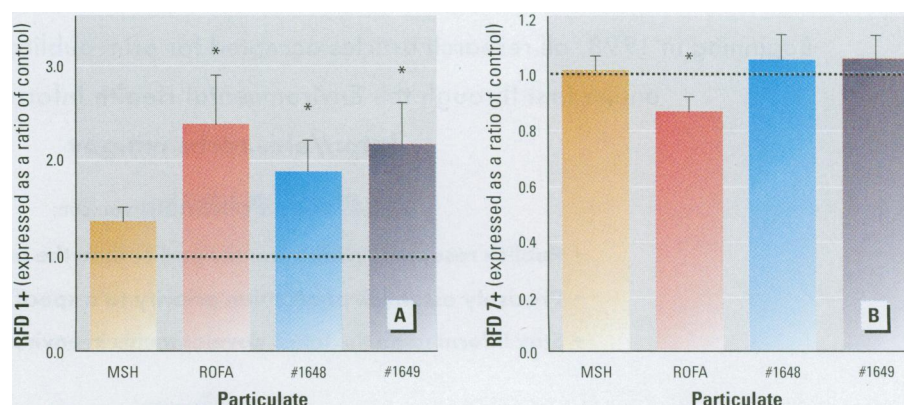


Figure 5. Changes in human alveolar macrophage phenotypes in response to residual oil fly ash (ROFA) and urban particles. Human alveolar macrophages were incubated with 25 $\mu\text{g}/\text{ml}$ ROFA, 100 $\mu\text{g}/\text{ml}$ of #1648 and #1649, or 200 $\mu\text{g}/\text{ml}$ Mount St. Helen's ash (MSH) for 24 hr and examined for surface expression of RFD1 and RFD7 epitopes by flow cytometry, as described in Materials and Methods. The data is expressed as the change relative to cells incubated without any particles (1 = 100% of control) for (A) RFD1 and (B) RFD1⁺7⁺. The data represents the mean \pm standard error ($n = 4$).

*Denotes either a significant increase or decrease ($p < 0.05$) in macrophage phenotype compared to control cells.

of increased symptoms in individuals with various chronic lung diseases and urban pollution.

In summary, urban particles, and to a greater extent ROFA particles, caused apoptosis of human AM, while MSH had no adverse effects. In addition, these particles shifted the ratio of AM phenotypes to a higher proinflammatory state. If these particles cause similar changes *in vivo*, this proinflammatory state may exacerbate pulmonary disease. Further studies will be needed to verify that the shift in AM phenotype occurs *in vivo* and is associated with increased inflammation.

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